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Invited review

Nucleotides in neuroregeneration and neuroprotection

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ABSTRACT

Brain injury generates the release of a multitude of factors including extracellular nucleotides, which exhibit bi-functional properties and contribute to both detrimental actions in the acute phase and also protective and reparative actions in the later recovery phase to allow neuroregeneration. A promising strategy toward restoration of neuronal function is based on activation of endogenous adult neural stem/progenitor cells. The implication of purinergic signaling in stem cell biology, including regulation of proliferation, differentiation, and cell death has become evident in the last decade. In this regard, current strategies of acute transplantation of ependymal stem/progenitor cells after spinal cord injury restore altered expression of P2X4 and P2X7 receptors and improve functional locomotor recovery. The expression of both receptors is transcriptionally regulated by Sp1 factor, which plays a key role in the startup of the transcription machinery to induce regeneration-associated genes expression. Finally, general signaling pathways triggered by nucleotide receptors in neuronal populations converge on several intracellular kinases, such as PI3K/Akt, GSK3 and ERK1,2, as well as the Nrf-2/heme oxigenase-1 axis, which specifically link them to neuroprotection. In this regard, regulation of dual specificity protein phosphatases can become novel mechanism of actions for nucleotide receptors that associate them to cell homeostasis regulation.

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Contents

Abbreviations: (AIS), axon initial segment; (ATF3), activating transcription factor 3; (BBG), Brilliant Blue G; (BDNF), brain-derived neurotrophic factor; (bFGF), basic fibroblast growth factor; (BzATP), 3′-O-(4-benzoyl)benzoyl-ATP; (CaMKII), Ca^{2+}/calmodulin-dependent protein kinase II; (CNS), central nervous system; (CREB), cAMP response element-binding protein; (DINE), damage-induced neuronal endopeptidase; (DUSP), dual specificity protein phosphatase; (epSPCs), ependymal stem/progenitor cells; (epSPCis), ependymal stem/progenitor cells after injury; (ERK), extracellular signal-regulated kinases; (GSK3), glycogen synthase kinase 3; (ICAM-1), intercellular adhesion molecule 1; (IGF-I), insulin-like growth factor 1; (MAPK), mitogen-activated protein kinases; (miRNAs), microRNAs; (NFκB), nuclear factor κB; ($NMDG^+$), N-methyl-D-glucamine; (Nrf-2), nuclear factor erythroid 2-related factor 2; (NSPCs), neural stem/progenitor cells; (NTPDase), nucleoside triphosphate diphosphohydrolase; (PI3K), phosphoinositide 3-kinase; (PKC), protein kinase C; (RAGs), regeneration-associated genes; (SCI), spinal cord injury; (Sp1), specificity protein 1; (STAT3), signal transducer and activator of transcription 3; (SVZ), subventricular zone; (TNFα), tumor necrosis factor; (TrkB), tropomyosin receptor kinase B.

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1. Introduction

There are different situations, such as traumatic injury, neurodegenerative diseases or stroke events, which can compromise neuronal function, thus disrupting the dynamic equilibrium of the brain. In all these cases, brain repair is necessary and neuroregenerative mechanisms to restore neuronal function exist, although most of them still remain unveiled. In addition, the hypothesis that neurodegeneration is a failure of neuroregeneration, increases the importance and complexity of the regenerative process (Armstrong and Barker, 2001; Enciu et al., 2011; Gage and Temple, 2013; Lim and Alvarez-Buylla, 2014). It is to emphasize that a broad heterogeneity among adult neural stem cells exists, their regional specification emerging in early embryonic development. The diverse and fine-tuned molecular and biochemical properties of stem cell types are essential for specific neurorepair, but it is also part of the difficulty for a feasible clinical application (Fuentealba et al., 2015; Merkle et al., 2014). There is not a clear frontier between neuroregeneration and neuroprotection as many compounds employed with neuroprotective purposes have also been described as factors inducing neuronal proliferation and differentiation. Thus, neuroprotection can be defined as the mechanisms trying to reduce secondary side effects of a large variety of internal or external aggressions that require displaying specific mechanisms for survival of neural cells. Among these aggressions, attention will be paid to glutamate NMDA receptor-induced excitotoxicity, oxidative stress, genotoxic stress, decrease in brain neurotrophins or estradiol and other neuroactive steroids (Arevalo et al., 2015; Garcia-Yague et al., 2013; Hansen et al., 2002; Lastres-Becker et al., 2014; Sofroniew et al., 2001). However, in spite of the great advances in these areas, only a small fraction of their possibilities are already known.

Since the beginning, extracellular nucleotides have proved to be a solid evidence of playing a relevant role in neuroregeneration and neuroprotection. A deep knowledge of their biology and the signaling cascades mediated through P2 receptors activation is necessary to understand how these substances are involved in Central Nervous System (CNS) pathologies. Moreover, they offer an opportunity of promising new drug targets, expanding the boundaries of purinergic field (Abbracchio et al., 2009; Burnstock, 2008, 2015b; Koles et al., 2011; North and Jarvis, 2013; Zimmermann et al., 2012).

2. Involvement of purinergic receptors in CNS regeneration

CNS injuries caused by trauma, ischemia and stroke are complex disorders associated with massive loss of neurons, and constitute the principal cause of death and disability worldwide. Although current pharmacological treatments and rehabilitative therapies can improve neurological functions in some patients, it is frequent that CNS lesions result in at least some incurable impairment even with the best possible therapy. The main obstacle for neural repair is the limited regenerative capacity of injured neurons. Traumatic brain injury induce release of ATP from damaged astrocytes, neurons and endothelial cells, leading to the activation of purinergic receptors coupled to protein kinase cascades that regulate expression of genes implicated in long-term trophic actions, cell survival and cell growth (Neary et al., 2006, 2005). Synergistic actions of nucleotides and growth factors stimulate astrocyte proliferation, contributing to the process of reactive astrogliosis, which is crucial for restriction of brain injury and regeneration of nerves and glial cells in damaged area (Arthur et al., 2005; Franke, 2011; Ishibashi et al., 2006). Moreover, brain injuries caused by trauma or ischemia are also related to structural alterations of the axon initial segment (AIS), which is not only the site of action potential initiation but also plays an essential role in maintaining axon integrity and identity (Buffington and Rasband, 2011; Hedstrom et al., 2008; Kole et al., 2008). In physiological conditions, AIS plasticity is controlled by the influx of calcium through voltage-gated calcium channels or glutamate receptors (Evans et al., 2013). However, AIS disruption following middle cerebral artery occlusion-induced brain ischemia takes place by a calcium-dependent mechanism independent of NMDA receptors that can be prevented by antagonism of P2X7 receptors (Del Puerto et al., 2015). Furthermore, suppression or inhibition of P2X7 receptor promotes axonal growth, suggesting a role of P2X7 antagonists in axonal regeneration and axonal physiology (del Puerto et al., 2012; Diaz-Hernandez et al., 2008). In addition, P2X7 and possibly other purinergic receptors are emerging as important targets to interrupt status epilepticus. Thus, inhibition of P2X7 receptors decreases microglial activation and interleukin IL1β production as well as reduces seizures during status epilepticus induced by intra-amygdala microinjection of kainic acid (Engel et al., 2012). Noticeable, the combination of benzodiazepines and P2X7 antagonists results in almost complete interruption of seizures in a mice model of drug-resistant epilepsy (Engel et al., 2012).

2.1. Contribution of purinergic signaling in stem cell-based neuroregeneration

A promising strategy toward restoration of neuronal function is based on harnessing the innate mechanisms of the CNS for self-repair and regenerate through activation of endogenous neural stem/progenitor cells (NSPCs). NSPCs are multipotent cells that self-renew, readily expand *in vitro* forming colonies of undifferentiated cells called neurospheres, and are committed to the neural lineage (Reynolds and Weiss, 1992). The implication of purinergic signaling in stem cell biology, including regulation of proliferation, differentiation, and cell death has become evident in the last decade (Burnstock and Ulrich, 2011; Glaser et al., 2012; Ulrich et al., 2012). Purinergic receptors are expressed in both neural stem cells and neural progenitors from very early stages of frog, chick, and mammalian embryonic development, suggesting their participation in the regulation of proliferation and lineage specification

(Trujillo et al., 2009). Purinergic activation has been associated with proliferation and neurogenesis in neonatal and adult mouse olfactory epithelium (Jia and Hegg, 2010; Jia et al., 2009). Neurospheres obtained from fetal rat brain express P2X2-P2X7 receptor subunits, as well as $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors (Burnstock and Ulrich, 2011; Schwindt et al., 2011). Functional purinergic receptors have also been identified in the adult mammalian brain in specific neurogenic niches, the subventricular zone (SVZ) of the lateral ventricles and the subgranular layer of the hippocampal dentate gyrus (Shukla et al., 2005). Neurospheres derived from the adult mouse SVZ express P2X4 and P2X7 mRNA (Stafford et al., 2007), and have functional $P2Y_1$ and $P2Y_2$ receptors. Activation of these receptors increases cell proliferation in the presence of growth factors and stimulates the migration of neural progenitors isolated *in vitro* and expanded as neurospheres, which may be of relevance for the local movement of cells in the neurogenic niches (Grimm et al., 2010; Mishra et al., 2006; Scemes et al., 2003). Moreover, $P2Y_1$ receptor agonists reduce neurosphere size and neurosphere-forming frequency of primary neurospheres derived from adult mouse SVZ, allowing the possibility that a proportion of the neural progenitor cell population differentiates into neurons and glia (Stafford et al., 2007). The functionality of extracellular nucleotides is tightly controlled by ecto-nucleotidases, which are plasma membrane-bound enzymes with catalytic sites facing extracellular medium (Zimmermann et al., 2012). Nucleoside triphosphate diphosphohydrolase type 2 (NTPDase2), which catalyzes dephosphorylation of nucleoside triphosphates and diphosphates generating the respective nucleoside monophosphate, is highly expressed on adult neural progenitor cells of the SVZ and hippocampus but is absent on astrocytes or mature neurons (Braun et al., 2003; Shukla et al., 2005). Recent findings demonstrate that NTPDase2 plays a major role in controlling extracellular nucleotide concentrations in the neurogenic niches thereby acting as a homeostatic regulator of nucleotide-mediated neural progenitor cell proliferation and expansion under basal conditions (Gampe et al., 2015).

2.2. Role of purinergic signaling in novel spinal cord injury repair strategies

One of the major causes of physical disability worldwide produced by SNC lesion is the spinal cord injury (SCI), which involves an irreversible loss of function distal to the lesion (paralysis) as a result of axonal damage, demyelination, and death of oligodendrocytes, astrocytes, and neurons, including both spinal cord interneurons and motor neurons (Grossman et al., 2001; Hulsebosch, 2002). SCI occurs with a worldwide incidence of 15–40 cases per million people annually, which is more than 130,000 patients per year, and frequently affects to young adults (Furlan et al., 2009; Tator, 1995). Although no effective treatment currently exists for the mayor neurological deficits after SCI, there are several hopeful neuroprotective agents being investigated in ongoing clinical trials (Kwon et al., 2011; Tator et al., 2012). One of these promising treatment strategies consists of NSPCs transplantation for promoting spinal cord healing (Mothe and Tator, 2013). Ependymal stem/progenitor cells (epSPCs) are multipotent stem cells found in the periventricular region containing the central canal of the adult spinal cord (Ronaghi et al., 2010). Following SCI, epSPC progeny is recruited to the injury site, even when the injury does not affect the epSPCs or their processes, giving rise among others to new oligodendrocyte progenitor cells (Meletis et al., 2008). The intrinsic potential of epSPCs to replace some of the cells in the spinal cord after injury opens up the opportunity for developing non-invasive therapies for patients with SCI, through activating the differentiation of epSPCs into various cell types. An *in vitro* study in mice has shown that the manipulation of epSPCs after SCI might be a viable strategy for restoring neuronal dysfunction in humans. Thus, using specific differentiation protocol, 90% of differentiated cultures of epSPCs obtained after SCI stain positive for the motor-neuron-specific marker HB9, with 32% of these motor neurons displaying electrophysiological properties that resemble those of functional spinal motor neurons (Moreno-Manzano et al., 2009). Transplantation of adult spinal-cord derived neurospheres from healthy donors was first shown to slightly improve motor recovery with aberrant axonal sprouting associated with allodynia. However, acute transplantation of epSPCs cultured from spinal cord injured donors (ependymal stem/progenitor cells after injury, epSPCis) is able to efficiently reverse the paralysis associated with SCI in rats (Moreno-Manzano et al., 2009). Following SCI, large amounts of ATP and other nucleotides are released by the traumatized tissue leading to the activation of purinergic receptors that, in coordination with growth factors, induce lesion remodeling and repair (Burnstock and Ulrich, 2011; Glaser et al., 2012). On the other hand, blockade of purinergic receptors after SCI reduces the gliosis response and diminishes also the formation of a glial scar (Rodriguez-Zayas et al., 2012). A recent work shows that epSPCs express functional ionotropic P2X4 and P2X7, and metabotropic $P2Y_1$ and $P2Y_4$ receptors, able to respond to ATP, ADP, and other nucleotidic compounds (Gomez-Villafuertes et al., 2014). Furthermore, a comparative study between epSPCs cultured from healthy rats *versus* epSPCis, obtained from rats that one week earlier suffered an SCI, reveals that a downregulation of $P2Y_1$ receptor together with an upregulation of $P2Y_4$ receptor occur in epSPCis (Gomez-Villafuertes et al., 2015). These findings suggest that the expression levels of P2Y receptors may play a critical role in the modulation of neural progenitor cell expansion. In support of this idea, some reports demonstrate that purinergic signaling might be required not only for developmental progenitor cell expansion and neurogenesis, but also to maintain NSPCs niches in the adult brain. External addition of ATP or its analogs increases the mitotic index and rate of neural progenitor cells, whereas P2Y antagonists suppress both neurosphere expansion and the mitotic index of cells within those neurospheres (Lin et al., 2007). Remarkably, severe traumatic spinal cord contusion induces early and persistent increase in the expression of P2X4 and P2X7 receptors around the injury, which can be completely reversed by acute transplantation of undifferentiated epSPCis, correlating with a functional locomotor recovery in a rat model of SCI (Gomez-Villafuertes et al., 2015).

2.3. Nuclear factors involved in nerve regeneration

Injured neurons have the ability to switch the expression of regeneration-associated genes (RAGs) on and off according to the regeneration program (Raivich and Makwana, 2007; Sun and He, 2010). Numerous injury-inducible transcription factors have been identified to date, including c-Jun, sox11, CREB, Smad1, ATF3, AKRD1, NFIL3, p53, STAT3, C/EBPβ, and several KLF family members (Kiryu-Seo and Kiyama, 2011). However, it is required to know how such a large number of transcription factors interplay with each other to regulate RAGs synchronously, and how global and dynamic changes in gene expressions are controlled. In this line of thought, damage-induced neuronal endopeptidase (DINE) is considered to be an ideal representative gene to provide further information about how injury-inducible transcription factors cooperate with each other during nerve regeneration (Kiryu-Seo and Kiyama, 2004). The most intriguing property of DINE is its extreme transcriptional response against various kinds of peripheral and central nerve injuries, including motor, sensory and sympathetic nerve injuries, brain and spinal cord trauma, and brain ischemia (Boeshore et al., 2004; Kiryu-Seo et al., 2000). The enhanced

expression of DINE is strikingly restricted to neuronal cells, and not seen in glial cells. Co-expression of activating transcription factor 3 (ATF3), c-Jun, and signal transducer and activator of transcription 3 (STAT3) increased the DINE promoter activity, although these transcription factors do not directly bind to a specific element within the promoter. Instead, specificity protein 1 (Sp1) transcription factor directly binds with high affinity to GC-rich DNA sequences located in the region proximal to the transcription start site of the DINE promoter, functioning as a scaffolding protein to recruit c-Jun, ATF3, and STAT3 to elicit their functional synergy (Kiryu-Seo et al., 2008). Importantly, the mechanism by which Sp1 may provide c-Jun/ATF3/STAT3 with a platform would be practical and effective when increased gene expression is required in the event of a fatal emergency such as neuronal injury (Fig. 1). Sp1 is a multifunctional protein that, at the transcriptional level, is not induced in injured neurons, but is expressed constitutively. The accessibility of injury-inducible transcription factors is determined by the modification of Sp1, suggesting the possibility that different alterations of Sp1 recruit different injury-inducible transcription factors. The activity and stability of Sp1 is regulated at the protein level by post-translational modifications, including phosphorylation, acetylation, sumoylation, ubiquitination, and glycosylation (Hung et al., 2006; Tan and Khachigian, 2009). The activity of Sp1 is also modulated by interactions or interplay with other transcription factors such as p53, c-myc, Smad, AP-2, and E2F-1 (Wierstra, 2008). These additional transcription factors may participate in the Sp1/c-Jun/ATF3/STAT3 complex to yield maximum effects in injured neurons. Considering that the expression of some purinergic receptors implicated in CNS regeneration, such as P2X4 and P2X7 receptors, is transcriptionally regulated by Sp1 in basal conditions (Garcia-Huerta et al., 2012; Korenaga et al., 2001), we can speculate that nerve-injury transcription factors could be capable of enhancing P2X expression under pathological situations, similarly to what happens with RAGs. The majority of P2X4-positive cells following spinal cord lesion has been identified as activated microglia/macrophages and surviving neurons/neurites (Schwab et al., 2005). The expression of P2X4 receptors is also elevated in spinal cord microglia after peripheral nerve injury (Tsuda et al., 2003; Ulmann et al., 2008), and P2X4 knock-out mice have lower levels of neuroinflammation after SCI, resulting in significant improvement in tissue sparing and functional recovery, especially during the first week after injury (de Rivero Vaccari et al., 2012). Concerning P2X7 receptor, a post-ischemic, time-dependent upregulation of this receptor in neurons and glial cells has been demonstrated in a focal cerebral ischemia rat model, suggesting a key role for this receptor in the pathophysiology of cerebral ischemia *in vivo* (Franke et al., 2004). However, the relationship between P2X7 receptor expression and the pathogenesis of contusive SCI remains controversial. Some evidences indicate that blockade of P2X7 receptors improve both the functional and pathological consequences of SCI (Peng et al., 2009; Wang et al., 2004), whereas others conclude that P2X7 receptor antagonism fails to improve motor recovery or histopathological outcome (Marcillo et al., 2012). P2X7 receptor arouses special interest due to its dual function as an inhibitor of neurogenesis and axon outgrowth and inductor of cell death in other cases. In cultured hippocampal neurons and differentiated neuroblastoma cells axonal growth and branching is induced following P2X7 receptor inhibition (Fig. 2) (Diaz-Hernandez et al., 2008; Gomez-Villafuertes et al., 2009), whereas a strong activation of P2X7 receptor causes necrosis/apoptosis in both embryonic and adult neural progenitor cells (Delarasse et al., 2009; Messemer et al., 2013). Interestingly, it is suggested that cell death elicited by P2X7 receptor activation may counter-regulate progenitor cell survival after CNS injury, where excessive neuro- and gliogenesis is induced (Messemer et al., 2013).

3. Involvement of purinergic receptors in neuroprotection

In processes related to brain injury, nucleotide receptor activity accounts for changes and adaptations in different cell populations.

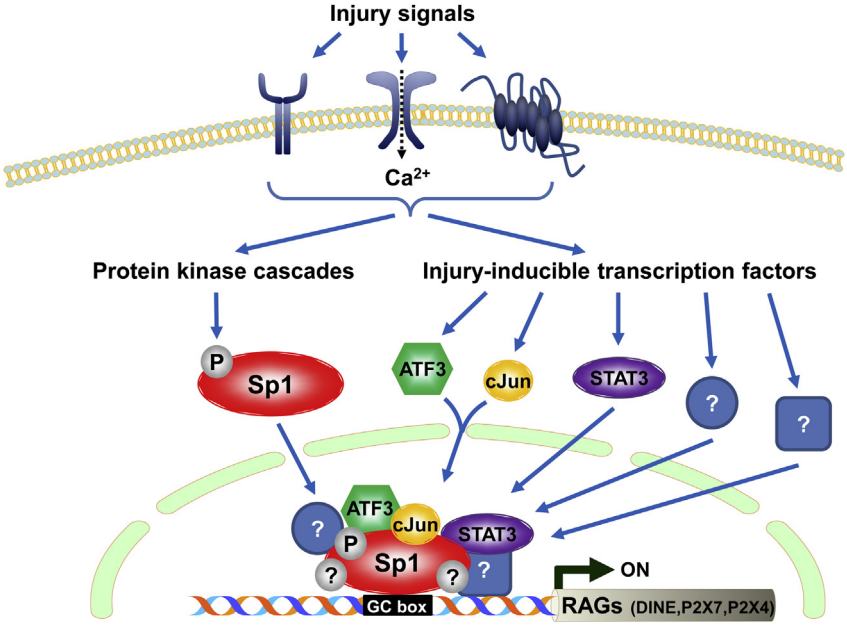

Fig. 1. Possible mechanism of Sp1-mediated transcriptional complex to induce the expression of regeneration-associated genes (RAGs) in response to nerve injury. Following nerve damage, brain/spinal cord trauma or cerebral ischemia, the injury-inducible transcription factor ATF3 binds to cJun and both enter the nucleus. There, Sp1 provides a platform to recruit the heterodimer ATF3/cJun and Stat3. The activity of Sp1 is regulated by post-translational modifications including phosphorylation, acetylation, sumoylation, ubiquitination, and glycosylation. Moreover, interaction with other transcription factors and chromatin modifiers participate and modulate the transcriptional machinery mediated by Sp1 that induces the expression of RAGs such as damage-induced neuronal endopeptidase (DINE) or some P2X receptor genes in response to nerve injury (adapted from Kiryu-Seo and Kiyama, 2011).

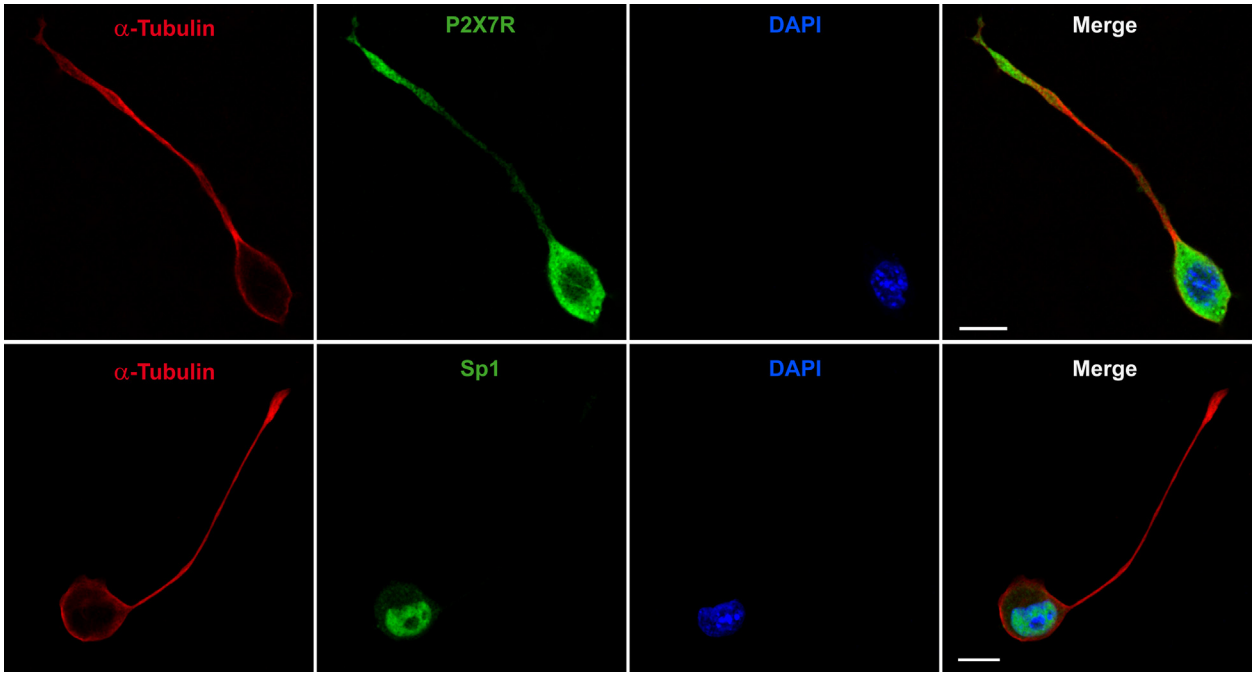

Fig. 2. Sp1-dependent expression of P2X7 receptor regulates axonal growth and branching in differentiated neuroblastoma cells. Immunofluorescences for P2X7 receptor (green, upper panel), Sp1 (green, lower panel) and α-tubulin (red) in Neuro-2a cell line cultured in serum-free medium for 72 h. Nuclei were counterstained with DAPI (blue). Merged channels are also shown. Confocal images were acquired with a TCS SPE microscope from Leica Microsystems with an Apochromat 63X, 1.3 NA oil immersion objective (Wetzlar, Germany). Scale bar = 15 μm.

In these conditions purinergic receptors act as primary sensors of ATP released. Until now, the increased purinergic signaling that follows CNS damage has been considered to contribute to development and maintenance of the subsequent inflammatory response. Therefore, it is not surprising that design of new purinergic ligands of therapeutic potential is usually based on antagonistic action on both P2X and P2Y receptors (Burnstock et al., 2011; Franke et al., 2012). Nevertheless, many examples of protective and trophic actions of purinergic agonists are emerging, adding new clues to the role of these receptors in animal models of neurodegenerative diseases, ischemic stroke and SCI (Chen and Brosnan, 2006; Delarasse et al., 2011; Hracsko et al., 2011; Marcillo et al., 2012; Monif et al., 2009). Moreover, purinergic receptors do not only mediate neuroprotection but also play active role in neurogenesis (Gampe et al., 2015; Ulrich and Illes, 2014). Therefore, it appears that some nucleotidic actions can also be crucial for preparing the required environmental conditions prior to subsequent processes of remodeling and repair once the inflammatory amplification phase has occurred. The overall data concerning these functions implies that special caution has to be taken when using drugs or pharmacological tools that systematically block these receptors. In fact, whether nucleotide receptor activation is beneficial or detrimental depends on a great deal of factors, including the nature of the toxic insult and the specific tissue cell population, both converging in a precise temporal window of the injury event.

3.1. Neuroprotective signaling triggered by P2X nucleotide receptors

Understanding of nucleotide neuroprotective role requires a model of study that provides a specific physiological niche in which controlled activation of nucleotide receptors lead to positive outcome. Primary cultures of cerebellar granule neurons represent one of the most popular *in vitro* models to study neuronal differentiation and survival. Indeed, cultured granule cells after 7 days *in vitro* acquire several electrophysiological and biochemical features of mature neurons (Burgoyne et al., 1993; Contestabile, 2002). In addition, trophic requirements operating for differentiation and maturation of cells in culture are the same than those occurring *in vivo* during development and migration of granule neurons. The most potent trophic factors, such as brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), and insulin-like growth factor 1 (IGF-I), trigger survival routes that mainly involve PI3K/Akt axis and MAPK activation. In addition, neuronal survival is highly dependent on synaptic activity and any signal promoting increase in intracellular calcium concentration is essential for the proper cell survival and differentiation (Contestabile, 2002). In this regard, different P2X and P2Y receptors expressed in these cells contribute to maintain survival conditions through their coupling to calcium signaling (Hervas et al., 2003; Sanchez-Nogueiro et al., 2009).

Among different nucleotidic receptors, P2X7 seems to play a relevant role in granule neuron physiology. In this respect, $[Ca^{2+}]_i$ measurements performed in mouse granule cell fibers in response to ATP or the P2X7 receptor agonist 3′-O-(4-benzoyl)benzoyl-ATP (BzATP) are ionotropic in nature and correspond to the characteristics of a P2X7 receptor (Sanchez-Nogueiro et al., 2009, 2005). Interestingly, the elusive responses to BzATP are only detectable when electrophysiological recordings are performed in cells bathed in a medium lacking Ca^{2+} and Mg^{2+}, therefore avoiding the inhibitory effect of extracellular divalent cations on P2X7 receptor activation (Coddou et al., 2011; North, 2002). Under these conditions, non-desensitizing currents triggered by BzATP administration correspond unequivocally to those caused by P2X7 receptors. In addition, BzATP also induces $[Ca^{2+}]_i$ elevations in both cell somata and axodendritic fibers of mouse granule neurons in stringent conditions of Mg^{2+}-free solution, being sensitive to Brilliant Blue G (BBG) and A-438079 specific P2X7 antagonists (Sanchez-Nogueiro et al., 2014). Accordingly, calcium transients induced by ATP in rat synaptic terminals isolated from adult rat cerebellum correlate well with responses exhibiting a

pharmacological profile of P2X7 receptor activation (Hervas et al., 2005). Overall, these results support the presence of functional P2X7 receptors with their peculiar features in cerebellar granule neurons.

3.1.1. P2X receptor coupling to glycogen synthase kinase 3 (GSK3) signaling. Interaction with growth factors

Studies on nucleotide receptors signaling reveal that both P2X and P2Y subtypes activate signaling cascades characteristic of trophic factors in granule neurons. In this model, ATP stimulation activates GSK3 phosphorylation at Ser9/21 residues (for β- and α-GSK3 isoforms, respectively) and inhibits its catalytic activity. This effect is characteristic of a low affinity ATP receptor, as GSK3 phosphorylation increased with ATP concentrations reaching the mM range. The receptor involved matches well with the pharmacological profile of a P2X7 receptor. Firstly, GSK3 phosphorylation is dependent on extracellular calcium concentration, fully activated by BzATP, and abolished in the presence of BBG and A-438079 (Ortega et al., 2009). Secondly, GSK3 phosphorylation is significantly potentiated when measured in medium lacking extracellular divalent cations such as Ca^{2+} and Mg^{2+} (Coddou et al., 2011; North, 2002).

In primary cultures of cerebellar granule neurons, the inhibition of GSK3 activity induced by P2X7 receptors promotes neuroprotection. Indeed, GSK3 activity is toxic for neurons, as many GSK3 substrates are active members of the mitochondrial apoptotic cascade, such as the protein Bax (Hetman et al., 2000; Linseman et al., 2004). Therefore, any trophic signal coupled to GSK3 phosphorylation and inhibition presents pro-survival activity (Chin et al., 2005). Unexpectedly, P2X7 receptors provide a novel mechanism for GSK3 inactivation that is dependent on protein kinase C (PKC) and by-pass the classical phosphoinositide 3-kinase (PI3K)/Akt-dependent route used by growth factors (Ortega et al., 2009). Moreover, this activity conveys an alternative pathway displayed by P2X7 receptors to maintain survival in conditions of inactivation of the main PI3K/Akt axis. This apoptotic paradigm can occur during trophic factor deprivation, and can be experimentally mimicked through pharmacological inhibition of PI3K activity with the compound LY294002 (D'Mello et al., 1993; Hetman et al., 2000; Miller et al., 1997). In this regard, under conditions of exposure to LY294002, the stimulation of P2X7 receptors rescues granule cells from apoptosis and prevents the increase in caspase-3 and GSK3 catalytic activity (Ortega et al., 2009). This neuroprotective effect requires GSK3 phosphorylation *via* PKC, specifically calcium-dependent PKC subtypes expressed in granule neurons (Popp et al., 2006). In other cell populations, such as cortical astrocytes, GSK3 signaling mediated by different nucleotide receptors also follows the PKC-dependent pathway (Neary and Kang, 2006). In contrast to the granule neurons scenario, nucleotides acting at P2X7 receptors are able to couple to canonical PI3K/Akt signaling, which can promote survival and proliferation of cortical astrocytes after mechanical strain caused injury (Neary et al., 2005). Other trophic signals in granule neurons, such as BDNF and moderate stimulation of NMDA receptors are also coupled to either PI3K or PKC to phosphorylate and inhibit GSK3 activity, therefore contributing to neuronal survival in conditions of PI3K inactivation (Fig. 3) (Ortega et al., 2010; Zirrgiebel et al., 1995). Similarly, sub-maximal activation of NMDA receptors in cortical neurons also elicits survival by maintaining inhibition of GSK3 activity through PI3K independent mechanisms (Habas et al., 2006). These results indicate that alternative signaling routes can be triggered to promote survival when the main pro-survival PI3K/Akt pathway is non-functional and nucleotides released to the extracellular space behave as important signals to assure minimum levels of survival.

As previously mentioned, GSK3 acts as a convergence point for several pro-survival factors that assure fine tuning of GSK3 activity (Chin et al., 2005). Interestingly, positive interactions of P2X7 receptors with NMDA and BDNF receptors also occur in granule

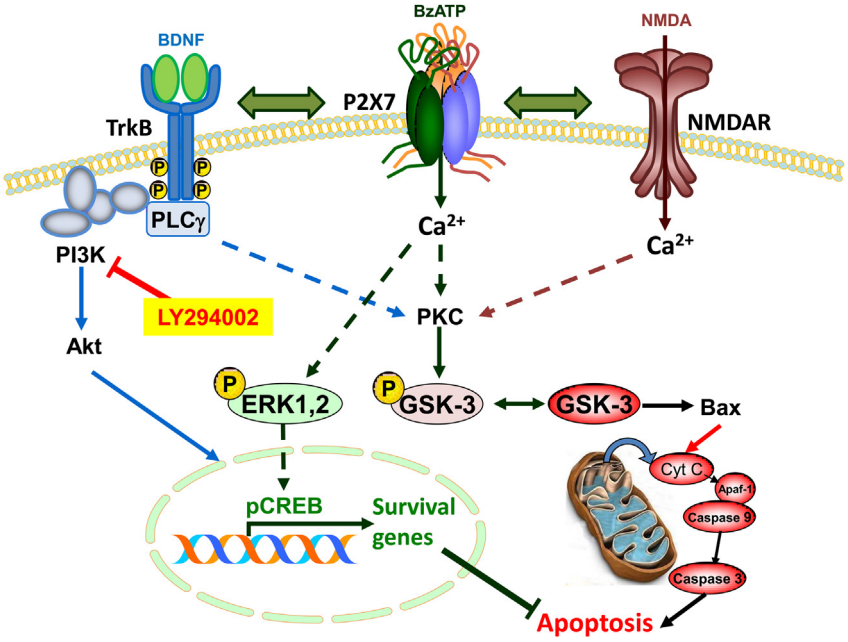

Fig. 3. P2X7 receptor mediated signaling associated to neuroprotection. Signaling activated by P2X7 receptors in granule neurons links to calcium-dependent events. The activation of ERK1,2-MAPK *via* Ca^{2+}-CaMKII provides a route of CREB phosphorylation and activation, which mediates transcription of pro-survival genes. This pathway is responsible of protection against glutamate-induced excitotoxicity. In addition, a novel mechanism of GSK3 phosphorylation involves calcium dependent-PKCs that by-pass the canonical PI3K/Akt/GSK3 axis classically triggered by growth factors. This mechanism of action allows P2X7 receptor to maintain survival in conditions of PI3K inactivation. As well, TrkB and NMDA receptors also present the ability to couple to PKC/GSK3 alternative route when the usual PI3K/Akt axis is not functional. Additionally, P2X7 receptors exhibit a priming effect by enhancing survival when combined with BDNF and NMDA receptor stimulation.

neurons that potentiate survival response against apoptosis induced by PI3K inactivation. In agreement with this, both P2X7 and NMDA receptors exhibit some degree of synergism on GSK3 phosphorylation (Fig. 3) (Ortega et al., 2010). This positive interplay already described for P2X7 receptors reaches particular relevance in granule neurons, providing alternative routes to support cell survival in conditions of limiting growth factor availability or reduced synaptic activity.

It is to notice that functional relationship between NMDA and BDNF receptors also occurs in granule neurons implying BDNF synthesis and release induced by NMDA receptor activation. Taking into account the similarities between the properties and behavior of NMDA and P2X7 receptors it cannot be excluded that P2X7 receptors could also participate in this autocrine loop connecting trophic and protective effects of BDNF to synaptic activity (Bazan-Peregrino et al., 2007; Marini et al., 1998; Zhu et al., 2002). In this support, several other examples in the literature concerns interaction between ionotropic P2X receptors and tropomyosin receptor kinase B (TrkB). Again, neuroprotective actions are attributed to P2X7 receptors on the basis of release of BDNF from Schwann cells (Verderio et al., 2006). In addition, in microglial cells from spinal cord, such level of cooperativity with BDNF has already been described for P2X4 receptors. In this occasion, through a Ca^{2+} and p38-dependent pathway, P2X4 receptors stimulates synthesis and release of BDNF, which behaves as a key molecule in microglia-neuron signaling and contributes to development of tactile allodynia (Trang et al., 2006; Tsuda et al., 2003).

3.1.2. P2X receptor coupling to mitogen-activated protein kinase (MAPK) signaling

As described for GSK3 phosphorylation, trophic activity of P2X7 receptor in granule neurons also accounts for coupling to extracellular signal-regulated kinases ERK1,2-MAPKs, Again, BzATP stimulates ERK1,2 phosphorylation with the pharmacological profile of a P2X7 receptor activation. In this case, the effect directly relates to P2X7-stimulated calcium signaling and is dependent on Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity (Leon et al., 2006; Ortega et al., 2011). Activation of ERK1,2 pathway contributes to survival against excitotoxic concentrations of glutamate (Fig. 3) (Ortega et al., 2011). Once more, BDNF shares with BzATP this pro-survival activity that is strongly dependent on the phosphorylation and activation of cAMP response element-binding protein (CREB), a well-known ERK1,2 target in granule neurons (Marini et al., 2004; Monti et al., 2002). In contrast, this survival pathway is stimulus-dependent, as P2X7 receptor-dependent ERK1,2 signaling does not protect from apoptosis induced by PI3K inactivation. These data support the notion that trophic signals as well as extracellular nucleotides can discriminate among distinctive transducing mechanisms to overcome different kinds of apoptotic insults (Hetman et al., 1999).

Another interesting cytoprotective role assigned for P2X7 receptors that involved the MAPK signaling is also ascribed to rat brain microglial cells. In this model, ATP activation of P2X7 receptors stimulates expression and release of tumor necrosis factor (TNFα). The presence of this cytokine in the extracellular medium seems to be beneficial as it contributes to the protection of surrounding neurons against glutamate-induced cell death (Hide et al., 2000; Suzuki et al., 2004).

3.2. Neuroprotective signaling triggered by P2Y nucleotide receptors

Concerning metabotropic P2Y receptors, extensive data exist in the literature that directly associate them to neuroprotective actions under different pathological conditions. Among them, $P2Y_2$ receptors behave as key players in astrocytic cells through the activation of multiple signaling cascades to elicit survival. PI3K-dependent activation of ERK, Akt and CREB signaling proteins plays a predominant role in survival after traumatic injury, contributing to upregulation of pro-survival proteins of the bcl family and downregulation of pro-apoptotic factors, such as Bad (Burgos et al., 2007; Chorna et al., 2004). In addition $P2Y_2$ receptors become upregulated during neuroinflammation and mediate protective and reparative functions through the activation of multiple intracellular pathways that involve growth factor receptor signaling cascades, nuclear factor κB (NFκB), MAPKs and tyrosine kinases in neuronal and glial cells (Weisman et al., 2012).

$P2Y_1$ receptors are also abundantly expressed in neurons and astrocytes of different brain regions and modulate important functions in pathophysiology. The activation of PI3K/Akt and MAPK signaling pathways are mediating processes like astrogliosis, proliferation and survival (Franke et al., 2012). Similarly, interleukin IL-6 released from astrocytes acts as a survival factor for hippocampal neurons submitted to oxidative stress (Fujita et al., 2009).

3.2.1. GSK3 and MAPK signaling routes in neuroprotection

Again, granule neurons provide evidence of P2Y receptor signaling coupled to neuroprotection. In this regard, PI3K/Akt/GSK3 pathway is targeted for a special type of ADP-P2Y responding receptor that is insensitive to $P2Y_1$ antagonists. The pharmacological profile for GSK3 phosphorylation induced by the non-hydrolyzable analog 2MeSADP in these cells corresponds to the activation of the more recently characterized $P2Y_{13}$ receptor, as was consistent with a Gi-coupled activity (Ortega et al., 2008). Of particular relevance is the novel association of the $P2Y_{13}$-coupled signaling pathway to the antioxidant Nrf-2/heme oxigenase-1 axis. This mechanism of action is dependent on GSK3 inhibition, which prevents negative regulation of the nuclear factor erythroid 2–related factor 2 (Nrf-2). Subsequent Nrf-2 nuclear accumulation regulates transcription of phase-2 antioxidant genes (Rada et al., 2012; Rojo et al., 2008). This pathway directly links $P2Y_{13}$ receptors to neuroprotection against oxidative stress (Espada et al., 2010). In agreement with these results, $P2Y_{13}$-mediated GSK3 inhibition also prevents phosphorylation and degradation of the GSK3 substrate, β-catenin. This transcriptional regulator can then accumulate into the nucleus and induce the expression of several genes involved in survival and differentiation (Fig. 4) (Ortega et al., 2008). In addition, $P2Y_{13}$ receptors also activate ERK1,2-dependent signaling and prevent cell death and caspase-3 activation in conditions of glutamate induced excitotoxicity. Again, the phosphorylation of CREB transcription factor plays an essential role in this pro-survival effect (Fig. 4) (Ortega et al., 2011). ADP metabotropic signaling through $P2Y_1$ and $P2Y_{13}$ receptors has also been reported in different populations of cerebellar astrocytes. In both neurons and astrocytes, GSK3 and ERK-1,2 signaling seems to be specifically triggered by $P2Y_{13}$ receptors that mediate trophic and pro-survival effects and exceed the role and function of $P2Y_1$-mediated activity (Perez-Sen et al., 2015).

3.2.2. Relevant role of dual specificity protein phosphatases (DUSP) in neuroprotection

As mentioned above, neuroprotective role for nucleotide receptors deals with direct action on key kinase cascades. Novel signaling mechanisms are emerging, which involve protein phosphatases as new targets for nucleotide receptors and connect them to cell homeostasis and explain some protective effects.

In this regard, a new role for $P2Y_{13}$ receptors in granule neurons conveys the regulation of expression of a particular member of dual specificity protein phosphatases, DUSP2, which sequentially recognize both Ser/Thr and Tyr residues. Namely, DUSP2 belongs to

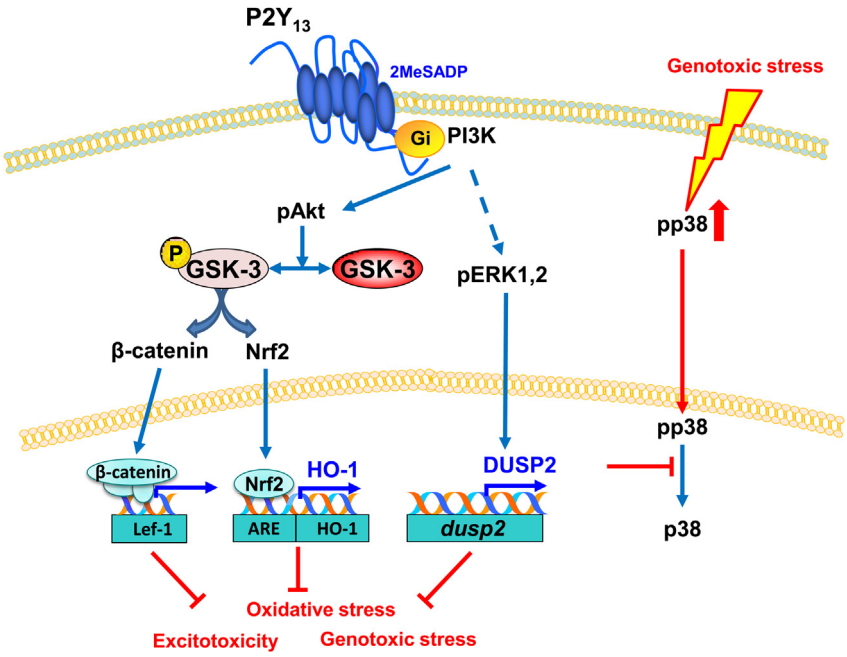

Fig. 4. $P2Y_{13}$ receptor mediated signaling associated to neuroprotection. $P2Y_{13}$ receptors promote neuroprotective actions through their coupling to classical survival kinases, such as GSK3 and ERK1/2, as well as protein phosphatases. $P2Y_{13}$ receptors activation of canonical PI3K/Akt/GSK3 pathway allows the cytosolic accumulation of the GSK3 substrates, β-catenin and the transcription factor Nrf-2. Both translocate to the nucleus where they regulate the expression of genes related to anti-apoptotic actions. Specially, the expression of heme-oxigenase-1 (HO-1) explains the protective role of $P2Y_{13}$ against oxidative stress. In addition, $P2Y_{13}$ receptors also activate ERK1,2 signaling in a way dependent on PI3K activity. Among different proteins, dual specificity protein phosphatase-2 (DUSP2) resulted to be a specific target of gene expression activated by ERK1,2. The latter represents a novel mechanism of action by which $P2Y_{13}$ receptors participate in negative feedback regulation of MAPK signaling. In line with this, they assure recovery of basal levels of phosphor-p38 increased in response to genotoxic stress. Therefore, $P2Y_{13}$ receptors can function as essential players in cell homeostasis and survival in neurons.

the subfamily of phosphatases that specifically dephosphorylate MAP kinases as substrates (MKPs, MAPK phosphatases). According to its behavior as an immediate-early gene, *dusp2* is rapidly induced and its expression depends on ERK1,2-mediated signaling stimulated by $P2Y_{13}$ receptors. This mechanism of action allows $P2Y_{13}$ receptors to participate in feedback negative regulation of MAPK signaling in neurons. In addition, regulation of DUSP2 expression contributes to the pro-survival activity of $P2Y_{13}$ receptors against genotoxic stress induced by cisplatin (Fig. 4). Cytotoxic action of this drug increases phosphorylated levels of p38 stress-related MAPK, which steadily accumulates into the nucleus. The time-course of DUSP2 protein expression elicited by $P2Y_{13}$ receptor activation correlates well with the recovery of low basal levels of the phosphorylated form of nuclear p38 (Fig. 5) (Morente et al., 2014).

While $P2Y_{13}$ receptors seem to play a fundamental role in cerebellar cells, in cortical astrocytes another ADP responding receptor $P2Y_1$ functions to regulate the expression of protein tyrosine phosphatase. It is to notice that this signaling mechanism contributes to suppress ERK1,2 hyper-activation elicited by oxidative stress and in addition to protect against cell death (Shinozaki et al., 2005, 2006).

The regulation of protein phosphatase activities and expression by nucleotide $P2Y_1$ and $P2Y_{13}$ receptors opens new insights into the function of nucleotide receptors as main regulators of cell signaling homeostasis. Therefore, they can have potential implications in disorders characterized by marked alterations of MAPK signaling, such as ischemia, neurological disorders, as well as tumor progression and metastatic processes. In addition, this new role supports the general view that ADP-P2Y receptors exert a protective role in several cell populations. The regulation of diverse protein phosphatase families by nucleotide receptors can be a general mechanism of action that extends their pro-survival activity in different models. Future work will be necessary to fully understand the potential role of protein phosphatases, mainly the family of dual specificity phosphatases, as new pharmacological targets.

3.3. *Physiological meaning of nucleotide receptor signaling in neurons*

All data reviewed here support the role of nucleotide receptors displaying trophic signaling to maintain proper functioning and survival in cell models of CNS. Among different mechanisms, GSK3 and ERK1,2 signaling pathways are gaining particular relevance and associate nucleotide receptors with neuroprotective actions.

At this point, it is necessary to emphasize that other studies have reported toxic actions for ATP and other nucleotide analogs in different neuronal and glial populations (Volonte et al., 2003). Specific culture conditions can provide distinct maturation stages with a particular environmental niche, which greatly influences receptors expression, heteromer formation and their coupling to diverse signaling partners. Besides environmental factors, nucleotide receptor isoforms can account for these apparent discrepancies. This is particularly striking for P2X7 receptors in which a high degree of heterogeneity has been found in mediated responses and final outcome (Sperlagh and Illes, 2014). Not only P2X7 receptors are highly polymorphic and revealed both loss- and gain-of function activities among the different forms, but also several mouse and human splice variants have been reported that include some truncated isoforms coexisting in the same cell, and some of them can escape from genetic knock-down of transgenic models. Moreover, it cannot be excluded that notable differences at the level of P2X7 receptor density in different strains of rodent species can account for opposing effects (Adinolfi et al., 2010; Cheewatrakoolpong et al., 2005; Masin et al., 2012; Miller et al., 2011; Nicke et al., 2009; Sluyter and Stokes, 2011). As an example,

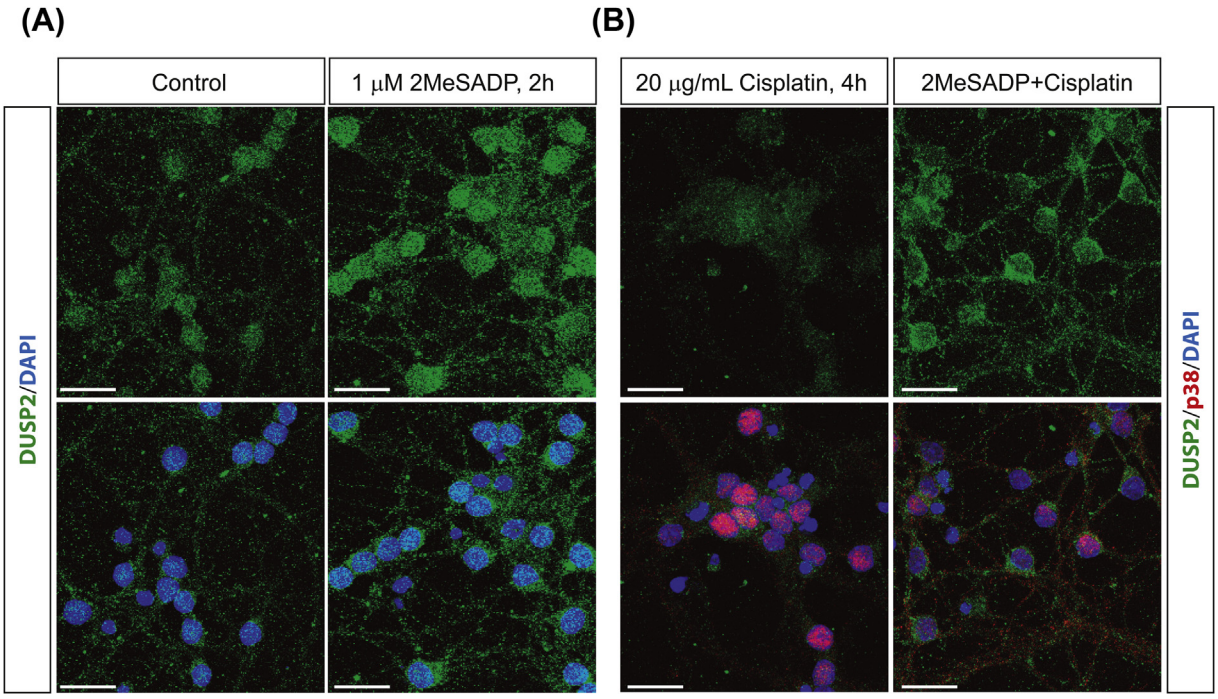

Fig. 5. Dual specificity protein phosphatase activation by $P2Y_{13}$ receptors in neurons. A) Immunofluorescence images depicts an increased expression of the nuclear inducible DUSP2 protein phosphatase (green) after $P2Y_{13}$ receptor activation by 2MeSADP analog in granule neurons, and merge with DAPI nuclear dye (blue). B) Confocal images of DUSP2 (green) and phosphor-p38 (red) staining of granule neurons show rapid accumulation of phosphor-p38 at the nucleus when exposed to genotoxic stress induced by cisplatin. The increased expression of DUSP2 phosphatase triggered by 2MeSADP decreases phosphor-p38 levels at the nucleus counterstained with DAPI (blue). Confocal images were obtained with a TCS SPE microscope from Leica Microsystems with a 20X objective (Wetzlar, Germany). Scale bar = 20 μm.

we have found out remarkable species differences between rat and mouse P2X7 responses, concerning the sensitivity to ATP, the physiological agonist, the magnitude of ionic currents elicited by BzATP, and other cell changes produced after prolonged stimulation of P2X7 receptors. The magnitude and current density of the non-desensitizing currents elicited by nucleotides in mouse cerebellar astrocytes are higher than those from rat cerebellar astrocytes. Another distinctive feature of BzATP currents from mouse cerebellar astrocytes is the progressive increase in the fractional permeability to N-methyl-D-glucamine ($NMDG^+$) during prolonged nucleotide stimulation. In fact, sustained BzATP application induces a gradual increase in membrane permeability to large cations, such as $NMDG^+$ and Yo-Pro-1, which ultimately led to the death of mouse astrocytes. Unexpectedly, neither membrane permeability changes nor dye uptake occurs in rat cerebellar astrocytes under the same experimental conditions (Carrasquero et al., 2009; Salas et al., 2013). Instead, prolonged P2X7 stimulation in rat cerebellar astrocytes induced trophic actions (Carrasquero et al., 2010). These differences probably could rely on the different expression levels of the P2X7 protein, the divergence in the sequence of the C-terminal tail region between the rat and mouse P2X7 receptor orthologues and on the accepted role of this intracellular domain in controlling plasma membrane permeability following P2X7 receptor stimulation (Costa-Junior et al., 2011; Chessell et al., 1998).

4. Future perspectives

As previously described, there is compelling evidence for a role of extracellular nucleotides in neuroregeneration and neuroprotection. However, this young and fertile field still offers a narrow view of all its potential. New technological approaches taking advantage of the "-omics" will expand our basic knowledge about biomedical relevance of nucleotides in brain functioning. Besides, new aspects of the cellular neurobiology of P2X and P2Y receptors need to be understood. Among them, the role of P2X4 in lysosomes and other subcellular organelles, the physiological meaning of the presence of P2X7 at the nuclear membrane or the location of some P2Y receptors in mitochondrial external membrane (Burnstock, 2015a). In addition, we have recently demonstrated the presence of P2X6 subunit into the cell nucleus, where it interacts with the splicing factor subunit SF3A1, modifying the mRNA splicing process (Diaz-Hernandez et al., 2015).

Another interesting aspect is related to the non-coding RNAs, including microRNAs (miRNAs), which can control mRNA stability and translation. miRNAs are very abundant, being unequally distributed among individual neurons, and can act as possible regulators of neuronal diversity and stability as well as modulators of axonal growth and branching (Han et al., 2011; Holt and Schuman, 2013; Kaplan et al., 2013). Currently, a few miRNA targeting purinergic P2 receptors expression have been described. miR-186 and miR-150 have been reported to down-regulate P2X7 expression in cancer epithelial cells, increasing cell viability (Zhou et al., 2008). Recently it has been found that activation of the $P2Y_2$ receptor can attenuate inflammation in endothelial cells through an increase in the expression of miR-22, which targets intercellular adhesion molecule 1 (ICAM-1) mRNA (Gidlof et al., 2015). A burst of scientific publications on miRNA acting on nucleotide neurobiology and their effects on neurorepair and neuroprotection are to be expected.

Epigenetic mechanisms, responsible for embryonic development till the final phenotype of neuron subtypes has been acquired, and the plastic changes in the adult CNS are now in the very front of Neuroscience. A first approach has been made trying to identify the different DNA methylation pattern in the brain of Alzheimer's disease patients and genetically modified animals (Sanchez-Mut et al., 2013). However, there is no data available on the involvement of

DNA methylation in the control of the expression of purinergic receptors, or other purinergic neurobiology components, in neurodegenerative diseases or when neurorepair and neuroprotection are necessary. From this perspective, the purinergic field is a fertile area expanding its boundaries.

Conflict of interest

Authors declare no conflict of interest in the present manuscript.

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References

Abbracchio, M.P., Burnstock, G., Verkhratsky, A., Zimmermann, H., 2009. Purinergic signalling in the nervous system: an overview. Trends Neurosci. 32, 19–29.
Adinolfi, E., Cirillo, M., Woltersdorf, R., Falzoni, S., Chiozzi, P., Pellegatti, P., Callegari, M.G., Sandona, D., Markwardt, F., Schmalzing, G., Di Virgilio, F., 2010. Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor. FASEB J. 24, 3393–3404.
Arevalo, M.A., Azcoitia, I., Garcia-Segura, L.M., 2015. The neuroprotective actions of oestradiol and oestrogen receptors. Nat. Rev. Neurosci. 16, 17–29.
Armstrong, R.J., Barker, R.A., 2001. Neurodegeneration: a failure of neuroregeneration? Lancet 358, 1174–1176.
Arthur, D.B., Akassoglou, K., Insel, P.A., 2005. P2Y2 receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. Proc. Natl. Acad. Sci. U. S. A. 102, 19138–19143.
Bazan-Peregrino, M., Gutierrez-Kobeh, L., Moran, J., 2007. Role of brain-derived neurotrophic factor in the protective action of N-methyl-D-aspartate in the apoptotic death of cerebellar granule neurons induced by low potassium. J. Neurosci. Res. 85, 332–341.
Boeshore, K.L., Schreiber, R.C., Vaccariello, S.A., Sachs, H.H., Salazar, R., Lee, J., Ratan, R.R., Leahy, P., Zigmond, R.E., 2004. Novel changes in gene expression following axotomy of a sympathetic ganglion: a microarray analysis. J. Neurobiol. 59, 216–235.
Braun, N., Sevigny, J., Mishra, S.K., Robson, S.C., Barth, S.W., Gerstberger, R., Hammer, K., Zimmermann, H., 2003. Expression of the ecto-ATPase NTPDase2 in the germinal zones of the developing and adult rat brain. Eur. J. Neurosci. 17, 1355–1364.
Buffington, S.A., Rasband, M.N., 2011. The axon initial segment in nervous system disease and injury. Eur. J. Neurosci. 34, 1609–1619.
Burgos, M., Neary, J.T., Gonzalez, F.A., 2007. P2Y2 nucleotide receptors inhibit trauma-induced death of astrocytic cells. J. Neurochem. 103, 1785–1800.
Burgoyne, R.D., Graham, M.E., Cambray-Deakin, M., 1993. Neurotrophic effects of NMDA receptor activation on developing cerebellar granule cells. J. Neurocytol. 22, 689–695.
Burnstock, G., 2008. Purinergic signalling and disorders of the central nervous system. Nat. Rev. Drug Discov. 7, 575–590.
Burnstock, G., 2015a. Intracellular expression of purinoceptors. Purinergic Signal 11 (3), 275–276.
Burnstock, G., 2015b. An introduction to the roles of purinergic signalling in neurodegeneration, neuroprotection and neuroregeneration. Neuropharmacology.
Burnstock, G., Krugel, U., Abbracchio, M.P., Illes, P., 2011. Purinergic signalling: from normal behaviour to pathological brain function. Prog. Neurobiol. 95, 229–274.
Burnstock, G., Ulrich, H., 2011. Purinergic signaling in embryonic and stem cell development. Cell Mol. Life Sci. 68, 1369–1394.
Carrasquero, L.M., Delicado, E.G., Bustillo, D., Gutierrez-Martin, Y., Artalejo, A.R., Miras-Portugal, M.T., 2009. P2X7 and P2Y13 purinergic receptors mediate intracellular calcium responses to BzATP in rat cerebellar astrocytes. J. Neurochem. 110, 879–889.
Carrasquero, L.M., Delicado, E.G., Sanchez-Ruiloba, L., Iglesias, T., Miras-Portugal, M.T., 2010. Mechanisms of protein kinase D activation in response to P2Y(2) and P2X7 receptors in primary astrocytes. Glia 58, 984–995.
Coddou, C., Yan, Z., Obsil, T., Huidobro-Toro, J.P., Stojilkovic, S.S., 2011. Activation and regulation of purinergic P2X receptor channels. Pharmacol. Rev. 63, 641–683.
Contestabile, A., 2002. Cerebellar granule cells as a model to study mechanisms of neuronal apoptosis or survival in vivo and in vitro. Cerebellum 1, 41–55.
Costa-Junior, H.M., Sarmento Vieira, F., Coutinho-Silva, R., 2011. C terminus of the P2X7 receptor: treasure hunting. Purinergic Signal 7, 7–19.
Cheewatrakoolpong, B., Gilchrest, H., Anthes, J.C., Greenfeder, S., 2005. Identification and characterization of splice variants of the human P2X7 ATP channel. Biochem. Biophys. Res. Commun. 332, 17–27.
Chen, L., Brosnan, C.F., 2006. Exacerbation of experimental autoimmune encephalomyelitis in P2X7R−/− mice: evidence for loss of apoptotic activity in lymphocytes. J. Immunol. 176, 3115–3126.
Chessell, I.P., Simon, J., Hibell, A.D., Michel, A.D., Barnard, E.A., Humphrey, P.P., 1998. Cloning and functional characterisation of the mouse P2X7 receptor. FEBS Lett. 439, 26–30.
Chin, P.C., Majdzadeh, N., D'Mello, S.R., 2005. Inhibition of GSK3beta is a common event in neuroprotection by different survival factors. Brain Res. Mol. Brain Res. 137, 193–201.
Chorna, N.E., Santiago-Perez, L.I., Erb, L., Seye, C.I., Neary, J.T., Sun, G.Y., Weisman, G.A., Gonzalez, F.A., 2004. P2Y(2) receptors activate neuroprotective mechanisms in astrocytic cells. J. Neurochem. 91, 119–132.
D'Mello, S.R., Galli, C., Ciotti, T., Calissano, P., 1993. Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. Proc. Natl. Acad. Sci. U. S. A. 90, 10989–10993.
de Rivero Vaccari, J.P., Bastien, D., Yurcisin, G., Pineau, I., Dietrich, W.D., De Koninck, Y., Keane, R.W., Lacroix, S., 2012. P2X4 receptors influence inflammasome activation after spinal cord injury. J. Neurosci. 32, 3058–3066.
del Puerto, A., Diaz-Hernandez, J.I., Tapia, M., Gomez-Villafuertes, R., Benitez, M.J., Zhang, J., Miras-Portugal, M.T., Wandosell, F., Diaz-Hernandez, M., Garrido, J.J., 2012. Adenylate cyclase 5 coordinates the action of ADP, P2Y1, P2Y13 and ATP-gated P2X7 receptors on axonal elongation. J. Cell Sci. 125, 176–188.
Del Puerto, A., Fronzaroli-Molinieres, L., Perez-Alvarez, M.J., Giraud, P., Carlier, E., Wandosell, F., Debanne, D., Garrido, J.J., 2015. ATP-P2X7 receptor modulates axon initial segment composition and function in physiological conditions and brain injury. Cereb. Cortex 25, 2282–2294.
Delarasse, C., Auger, R., Gonnord, P., Fontaine, B., Kanellopoulos, J.M., 2011. The purinergic receptor P2X7 triggers alpha-secretase-dependent processing of the amyloid precursor protein. J. Biol. Chem. 286, 2596–2606.
Delarasse, C., Gonnord, P., Galante, M., Auger, R., Daniel, H., Motta, I., Kanellopoulos, J.M., 2009. Neural progenitor cell death is induced by extracellular ATP via ligation of P2X7 receptor. J. Neurochem. 109, 846–857.
Diaz-Hernandez, J.I., Sebastian-Serrano, A., Gomez-Villafuertes, R., Diaz-Hernandez, M., Miras-Portugal, M.T., 2015. Age-related nuclear translocation of P2X6 subunit modifies splicing activity interacting with splicing factor 3A1. PLoS One 10, e0123121.
Diaz-Hernandez, M., del Puerto, A., Diaz-Hernandez, J.I., Diez-Zaera, M., Lucas, J.J., Garrido, J.J., Miras-Portugal, M.T., 2008. Inhibition of the ATP-gated P2X7 receptor promotes axonal growth and branching in cultured hippocampal neurons. J. Cell Sci. 121, 3717–3728.
Enciu, A.M., Nicolescu, M.I., Manole, C.G., Muresanu, D.F., Popescu, L.M., Popescu, B.O., 2011. Neuroregeneration in neurodegenerative disorders. BMC Neurol. 11, 75.
Engel, T., Gomez-Villafuertes, R., Tanaka, K., Mesuret, G., Sanz-Rodriguez, A., Garcia-Huerta, P., Miras-Portugal, M.T., Henshall, D.C., Diaz-Hernandez, M., 2012. Seizure suppression and neuroprotection by targeting the purinergic P2X7 receptor during status epilepticus in mice. FASEB J. 26, 1616–1628.
Espada, S., Ortega, F., Molina-Jijon, E., Rojo, A.I., Perez-Sen, R., Pedraza-Chaverri, J., Miras-Portugal, M.T., Cuadrado, A., 2010. The purinergic P2Y(13) receptor activates the Nrf2/HO-1 axis and protects against oxidative stress-induced neuronal death. Free Radic. Biol. Med. 49, 416–426.
Evans, M.D., Sammons, R.P., Lebron, S., Dumitrescu, A.S., Watkins, T.B., Uebele, V.N., Renger, J.J., Grubb, M.S., 2013. Calcineurin signaling mediates activity-dependent relocation of the axon initial segment. J. Neurosci. 33, 6950–6963.
Franke, H., 2011. Role of G protein-coupled receptors (GPCRs) for purines and pyrimidines in mediating degeneration and regeneration under neuroinflammatory processes. Purinergic Signal 7, 1–5.
Franke, H., Gunther, A., Grosche, J., Schmidt, R., Rossner, S., Reinhardt, R., Faber-Zuschratter, H., Schneider, D., Illes, P., 2004. P2X7 receptor expression after ischemia in the cerebral cortex of rats. J. Neuropathol. Exp. Neurol. 63, 686–699.
Franke, H., Verkhratsky, A., Burnstock, G., Illes, P., 2012. Pathophysiology of astroglial purinergic signalling. Purinergic Signal 8, 629–657.
Fuentealba, L.C., Rompani, S.B., Parraguez, J.I., Obernier, K., Romero, R., Cepko, C.L., Alvarez-Buylla, A., 2015. Embryonic origin of postnatal neural stem cells. Cell 161, 1644–1655.
Fujita, T., Tozaki-Saitoh, H., Inoue, K., 2009. P2Y1 receptor signaling enhances neuroprotection by astrocytes against oxidative stress via IL-6 release in hippocampal cultures. Glia 57, 244–257.
Furlan, J.C., Kattail, D., Fehlings, M.G., 2009. The impact of co-morbidities on age-related differences in mortality after acute traumatic spinal cord injury. J. Neurotrauma 26, 1361–1367.
Gage, F.H., Temple, S., 2013. Neural stem cells: generating and regenerating the brain. Neuron 80, 588–601.
Gampe, K., Stefani, J., Hammer, K., Brendel, P., Potzsch, A., Enikolopov, G., Enjyoji, K., Acker-Palmer, A., Robson, S.C., Zimmermann, H., 2015. NTPDase2 and purinergic signaling control progenitor cell proliferation in neurogenic niches of the adult mouse brain. Stem Cells 33, 253–264.
Garcia-Huerta, P., Diaz-Hernandez, M., Delicado, E.G., Pimentel-Santillana, M., Miras-Portugal, M.T., Gomez-Villafuertes, R., 2012. The specificity protein factor Sp1 mediates transcriptional regulation of P2X7 receptors in the nervous system. J. Biol. Chem. 287, 44628–44644.
Garcia-Yague, A.J., Rada, P., Rojo, A.I., Lastres-Becker, I., Cuadrado, A., 2013. Nuclear import and export signals control the subcellular localization of Nurr1 protein in response to oxidative stress. J. Biol. Chem. 288, 5506–5517.
Gidlof, O., Sathanoori, R., Magistri, M., Faghihi, M.A., Wahlestedt, C., Olde, B., Erlinge, D., 2015. Extracellular uridine triphosphate and adenosine triphosphate

attenuate endothelial inflammation through miR-22-mediated ICAM-1 inhibition. J. Vasc. Res. 52, 71–80.
Glaser, T., Cappellari, A.R., Pillat, M.M., Iser, I.C., Wink, M.R., Battastini, A.M., Ulrich, H., 2012. Perspectives of purinergic signaling in stem cell differentiation and tissue regeneration. Purinergic Signal 8, 523–537.
Gomez-Villafuertes, R., del Puerto, A., Diaz-Hernandez, M., Bustillo, D., Diaz-Hernandez, J.I., Huerta, P.G., Artalejo, A.R., Garrido, J.J., Miras-Portugal, M.T., 2009. Ca2+/calmodulin-dependent kinase II signalling cascade mediates P2X7 receptor-dependent inhibition of neuritogenesis in neuroblastoma cells. FEBS J. 276, 5307–5325.
Gomez-Villafuertes, R., Rodriguez-Jimenez, F.J., Alastrue-Agudo, A., Stojkovic, M., Miras-Portugal, M.T., Moreno-Manzano, V., 2015. Purinergic receptors in spinal cord-derived ependymal stem/progenitor cells and its potential role in cell-based therapy for spinal cord injury. Cell Transpl. 24 (8), 1493–1509 (17).
Grimm, I., Ullsperger, S.N., Zimmermann, H., 2010. Nucleotides and epidermal growth factor induce parallel cytoskeletal rearrangements and migration in cultured adult murine neural stem cells. Acta Physiol. (Oxf.) 199, 181–189.
Grossman, S.D., Rosenberg, L.J., Wrathall, J.R., 2001. Temporal-spatial pattern of acute neuronal and glial loss after spinal cord contusion. Exp. Neurol. 168, 273–282.
Habas, A., Kharebava, G., Szatmari, E., Hetman, M., 2006. NMDA neuroprotection against a phosphatidylinositol-3 kinase inhibitor, LY294002 by NR2B-mediated suppression of glycogen synthase kinase-3beta-induced apoptosis. J. Neurochem. 96, 335–348.
Han, L., Wen, Z., Lynn, R.C., Baudet, M.L., Holt, C.E., Sasaki, Y., Bassell, G.J., Zheng, J.Q., 2011. Regulation of chemotropic guidance of nerve growth cones by microRNA. Mol. Brain 4, 40.
Hansen, H.H., Azcoitia, I., Pons, S., Romero, J., Garcia-Segura, L.M., Ramos, J.A., Hansen, H.S., Fernandez-Ruiz, J., 2002. Blockade of cannabinoid CB(1) receptor function protects against in vivo disseminating brain damage following NMDA-induced excitotoxicity. J. Neurochem. 82, 154–158.
Hedstrom, K.L., Ogawa, Y., Rasband, M.N., 2008. AnkyrinG is required for maintenance of the axon initial segment and neuronal polarity. J. Cell Biol. 183, 635–640.
Hervas, C., Perez-Sen, R., Miras-Portugal, M.T., 2003. Coexpression of functional P2X and P2Y nucleotide receptors in single cerebellar granule cells. J. Neurosci. Res. 73, 384–399.
Hervas, C., Perez-Sen, R., Miras-Portugal, M.T., 2005. Presence of diverse functional P2X receptors in rat cerebellar synaptic terminals. Biochem. Pharmacol. 70, 770–785.
Hetman, M., Cavanaugh, J.E., Kimelman, D., Xia, Z., 2000. Role of glycogen synthase kinase-3beta in neuronal apoptosis induced by trophic withdrawal. J. Neurosci. 20, 2567–2574.
Hetman, M., Kanning, K., Cavanaugh, J.E., Xia, Z., 1999. Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. J. Biol. Chem. 274, 22569–22580.
Hide, I., Tanaka, M., Inoue, A., Nakajima, K., Kohsaka, S., Inoue, K., Nakata, Y., 2000. Extracellular ATP triggers tumor necrosis factor-alpha release from rat microglia. J. Neurochem. 75, 965–972.
Holt, C.E., Schuman, E.M., 2013. The central dogma decentralized: new perspectives on RNA function and local translation in neurons. Neuron 80, 648–657.
Hracsko, Z., Baranyi, M., Csolle, C., Goloncser, F., Madarasz, E., Kittel, A., Sperlagh, B., 2011. Lack of neuroprotection in the absence of P2X7 receptors in toxin-induced animal models of Parkinson's disease. Mol. Neurodegener. 6, 28.
Hulsebosch, C.E., 2002. Recent advances in pathophysiology and treatment of spinal cord injury. Adv. Physiol. Educ. 26, 238–255.
Hung, J.J., Wang, Y.T., Chang, W.C., 2006. Sp1 deacetylation induced by phorbol ester recruits p300 to activate 12(S)-lipoxygenase gene transcription. Mol. Cell Biol. 26, 1770–1785.
Ishibashi, T., Dakin, K.A., Stevens, B., Lee, P.R., Kozlov, S.V., Stewart, C.L., Fields, R.D., 2006. Astrocytes promote myelination in response to electrical impulses. Neuron 49, 823–832.
Jia, C., Hegg, C.C., 2010. NPY mediates ATP-induced neuroproliferation in adult mouse olfactory epithelium. Neurobiol. Dis. 38, 405–413.
Jia, E.Z., Liang, J., Yang, Z.J., Zhu, T.B., Wang, L.S., Chen, B., Cao, K.J., Huang, J., Ma, W.Z., 2009. Smoking and coronary atherosclerosis: follow-up study in China. Clin. Exp. Pharmacol. Physiol. 36, 690–695.
Kaplan, B.B., Kar, A.N., Gioio, A.E., Aschrafi, A., 2013. MicroRNAs in the axon and presynaptic nerve terminal. Front. Cell Neurosci. 7, 126.
Kiryu-Seo, S., Kato, R., Ogawa, T., Nakagomi, S., Nagata, K., Kiyama, H., 2008. Neuronal injury-inducible gene is synergistically regulated by ATF3, c-Jun, and STAT3 through the interaction with Sp1 in damaged neurons. J. Biol. Chem. 283, 6988–6996.
Kiryu-Seo, S., Kiyama, H., 2004. DINE (damage induced neuronal endopeptidase). Protein Pept. Lett. 11, 451–460.
Kiryu-Seo, S., Kiyama, H., 2011. The nuclear events guiding successful nerve regeneration. Front. Mol. Neurosci. 4, 53.
Kiryu-Seo, S., Sasaki, M., Yokohama, H., Nakagomi, S., Hirayama, T., Aoki, S., Wada, K., Kiyama, H., 2000. Damage-induced neuronal endopeptidase (DINE) is a unique metallopeptidase expressed in response to neuronal damage and activates superoxide scavengers. Proc. Natl. Acad. Sci. U. S. A. 97, 4345–4350.
Kole, M.H., Ilschner, S.U., Kampa, B.M., Williams, S.R., Ruben, P.C., Stuart, G.J., 2008. Action potential generation requires a high sodium channel density in the axon initial segment. Nat. Neurosci. 11, 178–186.
Koles, L., Leichsenring, A., Rubini, P., Illes, P., 2011. P2 receptor signaling in neurons and glial cells of the central nervous system. Adv. Pharmacol. 61, 441–493.
Korenaga, R., Yamamoto, K., Ohura, N., Sokabe, T., Kamiya, A., Ando, J., 2001. Sp1-mediated downregulation of P2X4 receptor gene transcription in endothelial cells exposed to shear stress. Am. J. Physiol. Heart Circ. Physiol. 280, H2214–H2221.
Kwon, B.K., Okon, E., Hillyer, J., Mann, C., Baptiste, D., Weaver, L.C., Fehlings, M.G., Tetzlaff, W., 2011. A systematic review of non-invasive pharmacologic neuroprotective treatments for acute spinal cord injury. J. Neurotrauma 28, 1545–1588.
Lastres-Becker, I., Innamorato, N.G., Jaworski, T., Rabano, A., Kugler, S., Van Leuven, F., Cuadrado, A., 2014. Fractalkine activates NRF2/NFE2L2 and heme oxygenase 1 to restrain tauopathy-induced microgliosis. Brain 137, 78–91.
Leon, D., Hervas, C., Miras-Portugal, M.T., 2006. P2Y1 and P2X7 receptors induce calcium/calmodulin-dependent protein kinase II phosphorylation in cerebellar granule neurons. Eur. J. Neurosci. 23, 2999–3013.
Lim, D.A., Alvarez-Buylla, A., 2014. Adult neural stem cells stake their ground. Trends Neurosci. 37, 563–571.
Lin, J.H., Takano, T., Arcuino, G., Wang, X., Hu, F., Darzynkiewicz, Z., Nunes, M., Goldman, S.A., Nedergaard, M., 2007. Purinergic signaling regulates neural progenitor cell expansion and neurogenesis. Dev. Biol. 302, 356–366.
Linseman, D.A., Butts, B.D., Precht, T.A., Phelps, R.A., Le, S.S., Laessig, T.A., Bouchard, R.J., Florez-McClure, M.L., Heidenreich, K.A., 2004. Glycogen synthase kinase-3beta phosphorylates Bax and promotes its mitochondrial localization during neuronal apoptosis. J. Neurosci. 24, 9993–10002.
Marcillo, A., Frydel, B., Bramlett, H.M., Dietrich, W.D., 2012. A reassessment of P2X7 receptor inhibition as a neuroprotective strategy in rat models of contusion injury. Exp. Neurol. 233, 687–692.
Marini, A.M., Jiang, X., Wu, X., Tian, F., Zhu, D., Okagaki, P., Lipsky, R.H., 2004. Role of brain-derived neurotrophic factor and NF-kappaB in neuronal plasticity and survival: from genes to phenotype. Restor. Neurol. Neurosci. 22, 121–130.
Marini, A.M., Rabin, S.J., Lipsky, R.H., Mocchetti, I., 1998. Activity-dependent release of brain-derived neurotrophic factor underlies the neuroprotective effect of N-methyl-D-aspartate. J. Biol. Chem. 273, 29394–29399.
Masin, M., Young, C., Lim, K., Barnes, S.J., Xu, X.J., Marschall, V., Brutkowski, W., Mooney, E.R., Gorecki, D.C., Murrell-Lagnado, R., 2012. Expression, assembly and function of novel C-terminal truncated variants of the mouse P2X7 receptor: re-evaluation of P2X7 knockouts. Br. J. Pharmacol. 165, 978–993.
Meletis, K., Barnabe-Heider, F., Carlen, M., Evergren, E., Tomilin, N., Shupliakov, O., Frisen, J., 2008. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol. 6, e182.
Merkle, F.T., Fuentealba, L.C., Sanders, T.A., Magno, L., Kessaris, N., Alvarez-Buylla, A., 2014. Adult neural stem cells in distinct microdomains generate previously unknown interneuron types. Nat. Neurosci. 17, 207–214.
Messemer, N., Kunert, C., Grohmann, M., Sobottka, H., Nieber, K., Zimmermann, H., Franke, H., Norenberg, W., Straub, I., Schaefer, M., Riedel, T., Illes, P., Rubini, P., 2013. P2X7 receptors at adult neural progenitor cells of the mouse subventricular zone. Neuropharmacology 73, 122–137.
Miller, C.M., Boulter, N.R., Fuller, S.J., Zakrzewski, A.M., Lees, M.P., Saunders, B.M., Wiley, J.S., Smith, N.C., 2011. The role of the P2X(7) receptor in infectious diseases. PLoS Pathog. 7, e1002212.
Miller, T.M., Tansey, M.G., Johnson Jr., E.M., Creedon, D.J., 1997. Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I-mediated survival of cerebellar granule cells. J. Biol. Chem. 272, 9847–9853.
Mishra, S.K., Braun, N., Shukla, V., Fullgrabe, M., Schomerus, C., Korf, H.W., Gachet, C., Ikehara, Y., Sevigny, J., Robson, S.C., Zimmermann, H., 2006. Extracellular nucleotide signaling in adult neural stem cells: synergism with growth factor-mediated cellular proliferation. Development 133, 675–684.
Monif, M., Reid, C.A., Powell, K.L., Smart, M.L., Williams, D.A., 2009. The P2X7 receptor drives microglial activation and proliferation: a trophic role for P2X7R pore. J. Neurosci. 29, 3781–3791.
Monti, B., Marri, L., Contestabile, A., 2002. NMDA receptor-dependent CREB activation in survival of cerebellar granule cells during in vivo and in vitro development. Eur. J. Neurosci. 16, 1490–1498.
Moreno-Manzano, V., Rodriguez-Jimenez, F.J., Garcia-Rosello, M., Lainez, S., Erceg, S., Calvo, M.T., Ronaghi, M., Lloret, M., Planells-Cases, R., Sanchez-Puelles, J.M., Stojkovic, M., 2009. Activated spinal cord ependymal stem cells rescue neurological function. Stem Cells 27, 733–743.
Morente, V., Perez-Sen, R., Ortega, F., Huerta-Cepas, J., Delicado, E.G., Miras-Portugal, M.T., 2014. Neuroprotection elicited by P2Y13 receptors against genotoxic stress by inducing DUSP2 expression and MAPK signaling recovery. Biochim. Biophys. Acta 1843, 1886–1898.
Mothe, A.J., Tator, C.H., 2013. Review of transplantation of neural stem/progenitor cells for spinal cord injury. Int. J. Dev. Neurosci. 31, 701–713.
Neary, J.T., Kang, Y., 2006. P2 purinergic receptors signal to glycogen synthase kinase-3beta in astrocytes. J. Neurosci. Res. 84, 515–524.
Neary, J.T., Kang, Y., Shi, Y.F., Tran, M.D., Wanner, I.B., 2006. P2 receptor signalling, proliferation of astrocytes, and expression of molecules involved in cell-cell interactions. Nov. Found. Symp. 276, 131–143 discussion 143–137, 233–137, 275–181.
Neary, J.T., Kang, Y., Tran, M., Feld, J., 2005. Traumatic injury activates protein kinase B/Akt in cultured astrocytes: role of extracellular ATP and P2 purinergic receptors. J. Neurotrauma 22, 491–500.
Nicke, A., Kuan, Y.H., Masin, M., Rettinger, J., Marquez-Klaka, B., Bender, O., Gorecki, D.C., Murrell-Lagnado, R.D., Soto, F., 2009. A functional P2X7 splice

variant with an alternative transmembrane domain 1 escapes gene inactivation in P2X7 knock-out mice. J. Biol. Chem. 284, 25813–25822.
North, R.A., 2002. Molecular physiology of P2X receptors. Physiol. Rev. 82, 1013–1067.
North, R.A., Jarvis, M.F., 2013. P2X receptors as drug targets. Mol. Pharmacol. 83, 759–769.
Ortega, F., Perez-Sen, R., Delicado, E.G., Miras-Portugal, M.T., 2009. P2X7 nucleotide receptor is coupled to GSK-3 inhibition and neuroprotection in cerebellar granule neurons. Neurotox. Res. 15, 193–204.
Ortega, F., Perez-Sen, R., Delicado, E.G., Teresa Miras-Portugal, M., 2011. ERK1/2 activation is involved in the neuroprotective action of P2Y13 and P2X7 receptors against glutamate excitotoxicity in cerebellar granule neurons. Neuropharmacology 61, 1210–1221.
Ortega, F., Perez-Sen, R., Miras-Portugal, M.T., 2008. Gi-coupled P2Y-ADP receptor mediates GSK-3 phosphorylation and beta-catenin nuclear translocation in granule neurons. J. Neurochem. 104, 62–73.
Ortega, F., Perez-Sen, R., Morente, V., Delicado, E.G., Miras-Portugal, M.T., 2010. P2X7, NMDA and BDNF receptors converge on GSK3 phosphorylation and cooperate to promote survival in cerebellar granule neurons. Cell Mol. Life Sci. 67, 1723–1733.
Peng, W., Cotrina, M.L., Han, X., Yu, H., Bekar, L., Blum, L., Takano, T., Tian, G.F., Goldman, S.A., Nedergaard, M., 2009. Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after spinal cord injury. Proc. Natl. Acad. Sci. U. S. A. 106, 12489–12493.
Perez-Sen, R., Queipo, M.J., Morente, V., Ortega, F., Delicado, E.G., Miras-Portugal, M.T., 2015. Neuroprotection mediated by P2Y13 nucleotide receptors in neurons. Comput. Struct. Biotechnol. J. 13, 160–168.
Popp, R.L., Velasquez, O., Bland, J., Hughes, P., 2006. Characterization of protein kinase C isoforms in primary cultured cerebellar granule cells. Brain Res. 1083, 70–84.
Rada, P., Rojo, A.I., Evrard-Todeschi, N., Innamorato, N.G., Cotte, A., Jaworski, T., Tobon-Velasco, J.C., Devijver, H., Garcia-Mayoral, M.F., Van Leuven, F., Hayes, J.D., Bertho, G., Cuadrado, A., 2012. Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/beta-TrCP axis. Mol. Cell Biol. 32, 3486–3499.
Raivich, G., Makwana, M., 2007. The making of successful axonal regeneration: genes, molecules and signal transduction pathways. Brain Res. Rev. 53, 287–311.
Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710.
Rodriguez-Zayas, A.E., Torrado, A.I., Rosas, O.R., Santiago, J.M., Figueroa, J.D., Miranda, J.D., 2012. Blockade of P2 nucleotide receptors after spinal cord injury reduced the gliotic response and spared tissue. J. Mol. Neurosci. 46, 167–176.
Rojo, A.I., Rada, P., Egea, J., Rosa, A.O., Lopez, M.G., Cuadrado, A., 2008. Functional interference between glycogen synthase kinase-3 beta and the transcription factor Nrf2 in protection against kainate-induced hippocampal cell death. Mol. Cell Neurosci. 39, 125–132.
Ronaghi, M., Erceg, S., Moreno-Manzano, V., Stojkovic, M., 2010. Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? Stem Cells 28, 93–99.
Salas, E., Carrasquero, L.M., Olivos-Ore, L.A., Bustillo, D., Artalejo, A.R., Miras-Portugal, M.T., Delicado, E.G., 2013. Purinergic P2X7 receptors mediate cell death in mouse cerebellar astrocytes in culture. J. Pharmacol. Exp. Ther. 347, 802–815.
Sanchez-Mut, J.V., Aso, E., Panayotis, N., Lott, I., Dierssen, M., Rabano, A., Urdinguio, R.G., Fernandez, A.F., Astudillo, A., Martin-Subero, J.I., Balint, B., Fraga, M.F., Gomez, A., Gurnot, C., Roux, J.C., Avila, J., Hensch, T.K., Ferrer, I., Esteller, M., 2013. DNA methylation map of mouse and human brain identifies target genes in Alzheimer's disease. Brain 136, 3018–3027.
Sanchez-Nogueiro, J., Marin-Garcia, P., Bustillo, D., Olivos-Ore, L.A., Miras-Portugal, M.T., Artalejo, A.R., 2014. Subcellular distribution and early signalling events of P2X7 receptors from mouse cerebellar granule neurons. Eur. J. Pharmacol. 744, 190–202.
Sanchez-Nogueiro, J., Marin-Garcia, P., Leon, D., Leon-Otegui, M., Salas, E., Gomez-Villafuertes, R., Gualix, J., Miras-Portugal, M.T., 2009. Axodendritic fibres of mouse cerebellar granule neurons exhibit a diversity of functional P2X receptors. Neurochem. Int. 55, 671–682.
Sanchez-Nogueiro, J., Marin-Garcia, P., Miras-Portugal, M.T., 2005. Characterization of a functional P2X(7)-like receptor in cerebellar granule neurons from P2X(7) knockout mice. FEBS Lett. 579, 3783–3788.
Scemes, E., Duval, N., Meda, P., 2003. Reduced expression of P2Y1 receptors in connexin43-null mice alters calcium signaling and migration of neural progenitor cells. J. Neurosci. 23, 11444–11452.
Schwab, J.M., Guo, L., Schluesener, H.J., 2005. Spinal cord injury induces early and persistent lesional P2X4 receptor expression. J. Neuroimmunol. 163, 185–189.
Schwindt, T.T., Trujillo, C.A., Negraes, P.D., Lameu, C., Ulrich, H., 2011. Directed differentiation of neural progenitors into neurons is accompanied by altered expression of P2X purinergic receptors. J. Mol. Neurosci. 44, 141–146.
Shinozaki, Y., Koizumi, S., Ishida, S., Sawada, J., Ohno, Y., Inoue, K., 2005. Cytoprotection against oxidative stress-induced damage of astrocytes by extracellular ATP via P2Y1 receptors. Glia 49, 288–300.
Shinozaki, Y., Koizumi, S., Ohno, Y., Nagao, T., Inoue, K., 2006. Extracellular ATP counteracts the ERK1/2-mediated death-promoting signaling cascades in astrocytes. Glia 54, 606–618.
Shukla, V., Zimmermann, H., Wang, L., Kettenmann, H., Raab, S., Hammer, K., Sevigny, J., Robson, S.C., Braun, N., 2005. Functional expression of the ecto-ATPase NTPDase2 and of nucleotide receptors by neuronal progenitor cells in the adult murine hippocampus. J. Neurosci. Res. 80, 600–610.
Sluyter, R., Stokes, L., 2011. Significance of P2X7 receptor variants to human health and disease. Recent Pat. DNA Gene Seq. 5, 41–54.
Sofroniew, M.V., Howe, C.L., Mobley, W.C., 2001. Nerve growth factor signaling, neuroprotection, and neural repair. Annu. Rev. Neurosci. 24, 1217–1281.
Sperlagh, B., Illes, P., 2014. P2X7 receptor: an emerging target in central nervous system diseases. Trends Pharmacol. Sci. 35, 537–547.
Stafford, M.R., Bartlett, P.F., Adams, D.J., 2007. Purinergic receptor activation inhibits mitogen-stimulated proliferation in primary neurospheres from the adult mouse subventricular zone. Mol. Cell Neurosci. 35, 535–548.
Sun, F., He, Z., 2010. Neuronal intrinsic barriers for axon regeneration in the adult CNS. Curr. Opin. Neurobiol. 20, 510–518.
Suzuki, T., Hide, I., Ido, K., Kohsaka, S., Inoue, K., Nakata, Y., 2004. Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. J. Neurosci. 24, 1–7.
Tan, N.Y., Khachigian, L.M., 2009. Sp1 phosphorylation and its regulation of gene transcription. Mol. Cell Biol. 29, 2483–2488.
Tator, C.H., 1995. Update on the pathophysiology and pathology of acute spinal cord injury. Brain Pathol. 5, 407–413.
Tator, C.H., Hashimoto, R., Raich, A., Norvell, D., Fehlings, M.G., Harrop, J.S., Guest, J., Aarabi, B., Grossman, R.G., 2012. Translational potential of preclinical trials of neuroprotection through pharmacotherapy for spinal cord injury. J. Neurosurg. Spine 17, 157–229.
Trang, T., Beggs, S., Salter, M.W., 2006. Purinoceptors in microglia and neuropathic pain. Pflug. Arch. 452, 645–652.
Trujillo, C.A., Schwindt, T.T., Martins, A.H., Alves, J.M., Mello, L.E., Ulrich, H., 2009. Novel perspectives of neural stem cell differentiation: from neurotransmitters to therapeutics. Cytom. A 75, 38–53.
Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M.W., Inoue, K., 2003. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 424, 778–783.
Ulmann, L., Hatcher, J.P., Hughes, J.P., Chaumont, S., Green, P.J., Conquet, F., Buell, G.N., Reeve, A.J., Chessell, I.P., Rassendren, F., 2008. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. J. Neurosci. 28, 11263–11268.
Ulrich, H., Abbracchio, M.P., Burnstock, G., 2012. Extrinsic purinergic regulation of neural stem/progenitor cells: implications for CNS development and repair. Stem Cell Rev. 8, 755–767.
Ulrich, H., Illes, P., 2014. P2X receptors in maintenance and differentiation of neural progenitor cells. Neural Regen. Res. 9, 2040–2041.
Verderio, C., Bianco, F., Blanchard, M.P., Bergami, M., Canossa, M., Scarfone, E., Matteoli, M., 2006. Cross talk between vestibular neurons and Schwann cells mediates BDNF release and neuronal regeneration. Brain Cell Biol. 35, 187–201.
Volonte, C., Amadio, S., Cavaliere, F., D'Ambrosi, N., Vacca, F., Bernardi, G., 2003. Extracellular ATP and neurodegeneration. Curr. Drug Targets CNS Neurol. Disord. 2, 403–412.
Wang, X., Arcuino, G., Takano, T., Lin, J., Peng, W.G., Wan, P., Li, P., Xu, Q., Liu, Q.S., Goldman, S.A., Nedergaard, M., 2004. P2X7 receptor inhibition improves recovery after spinal cord injury. Nat. Med. 10, 821–827.
Weisman, G.A., Ajit, D., Garrad, R., Peterson, T.S., Woods, L.T., Thebeau, C., Camden, J.M., Erb, L., 2012. Neuroprotective roles of the P2Y(2) receptor. Purinergic Signal 8, 559–578.
Wierstra, I., 2008. Sp1: emerging roles–beyond constitutive activation of TATA-less housekeeping genes. Biochem. Biophys. Res. Commun. 372, 1–13.
Zhou, L., Qi, X., Potashkin, J.A., Abdul-Karim, F.W., Gorodeski, G.I., 2008. MicroRNAs miR-186 and miR-150 down-regulate expression of the pro-apoptotic purinergic P2X7 receptor by activation of instability sites at the 3'-untranslated region of the gene that decrease steady-state levels of the transcript. J. Biol. Chem. 283, 28274–28286.
Zhu, D., Lipsky, R.H., Marini, A.M., 2002. Co-activation of the phosphatidylinositol-3-kinase/Akt signaling pathway by N-methyl-D-aspartate and TrkB receptors in cerebellar granule cell neurons. Amino Acids 23, 11–17.
Zimmermann, H., Zebisch, M., Strater, N., 2012. Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 8, 437–502.
Zirrgiebel, U., Ohga, Y., Carter, B., Berninger, B., Inagaki, N., Thoenen, H., Lindholm, D., 1995. Characterization of TrkB receptor-mediated signaling pathways in rat cerebellar granule neurons: involvement of protein kinase C in neuronal survival. J. Neurochem. 65, 2241–2250.